HISTOLOGY 1 - FIRST COURSE

LAB -3- PREPARATION OF SLIDES, METHODS AND MATERIALS

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الجانب العملي محاضرة رقم 3

Practical Lecture No. 2

OUTLINES

1.Preparation of histological specimen.

2.Materials Used in Slide Preparation.

3.What is Rotary Microtome?

4.Procedure Using Rotary Microtome.

5.Application of Rotary Microtome.

Aims of lecture :-

1- TISSUE PRESERVATION

2- ACCURATE DIAGNOSIS

3- EXAMINATION PREPARATION

4- PREPARATION FOR ADVANCED EXAMINATIONS

5- PERMANENT FIXATION

Preparation of histological specimen.

1.Fixation using formalin to remove autolysis and bacterial attack

2.Washing using tab water to remove formalin

3.Dehydration to remove the water from the tissue because the water doesn t mix with the paraffin wax through embedding stage

4.Clearing replacing the dehydration fluid with xylene because the ethanol doesn t mix with paraffin wax

5.Embedding using paraffin wax as medium for keeping intact all parts of the tissue when sections are cut

6.Sectioning using microtome is a mechanical instrument used to cut biological specimens into very thin sections for microscopic examination

7.Staining of Paraffin Section:

A.Deparaffinization: Removal of wax is done by incubate at 65c for 30 minute and then using with xylene. It is essential to remove the wax completely.

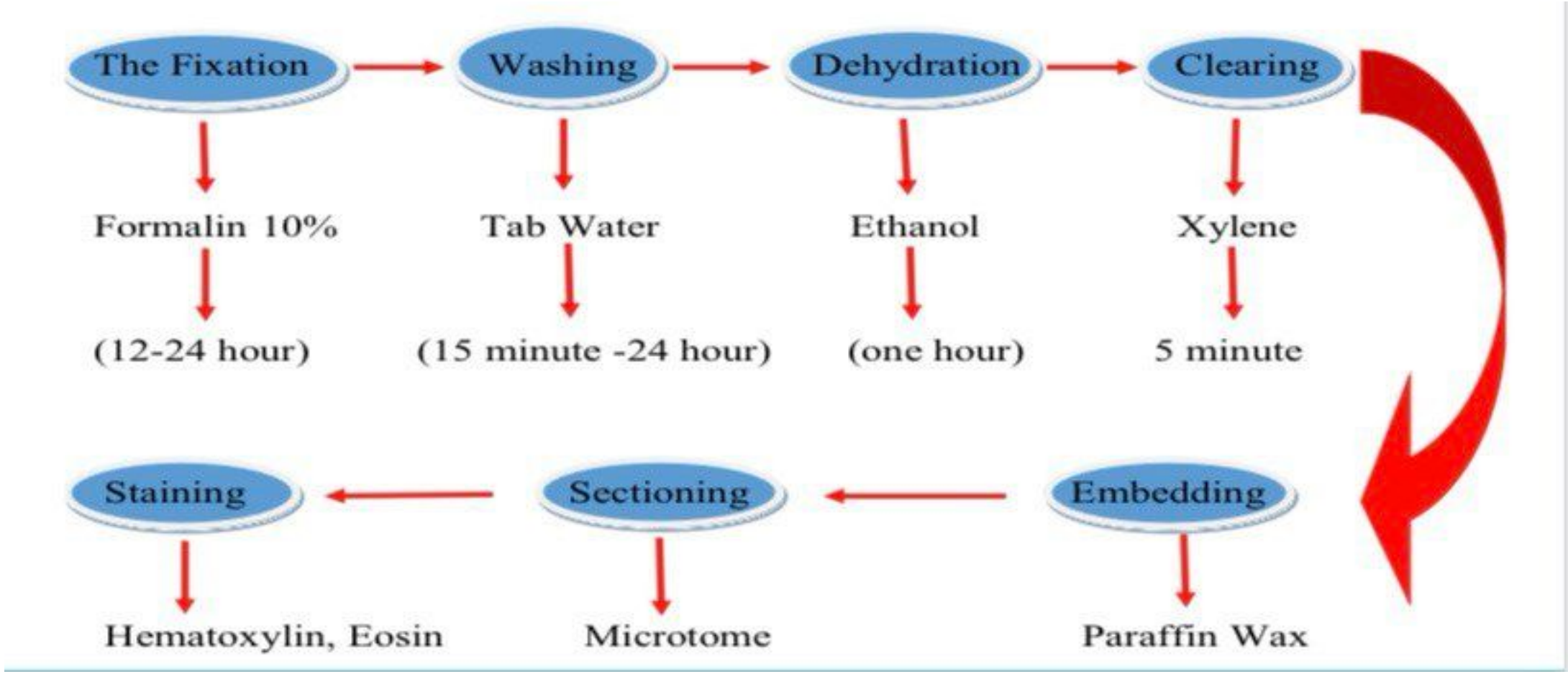
B.Hydration: The hydration is done with graded alcohols from higher concentration to lower **concentration.**

C. Staining: The most common stain applied for histological study is Hematoxylin and Eosin.

D. Dehydration and clearing: Dehydration is done in graded alcohols or acetones from 70% to absolute alcohol or acetone.

E. Cover slipping and mounting: The stained section on the slide must be covered with a thin piece of plastic or glass. Tissue sections must be mounted under a cover slip.

Preparation of Histological Specimen



Materials Used in Slide Preparation

1.Glass Slides and Coverslips

•Function: Provide support and protect the specimen.

2.Fixatives

•Examples: Formaldehyde, methanol, ethanol.

3.Dehydrating Agents

•Examples: Ethanol, isopropanol.

4.Clearing Agents

•Examples: Xylene, toluene

5.Embedding Media

•Examples: Paraffin wax, resin.

6.Stains

•Examples: Hematoxylin and Eosin, PAS, Wright Giemsa.

7.Mounting Media

•Examples: DPX, Canada Balsam.

8.Microtome

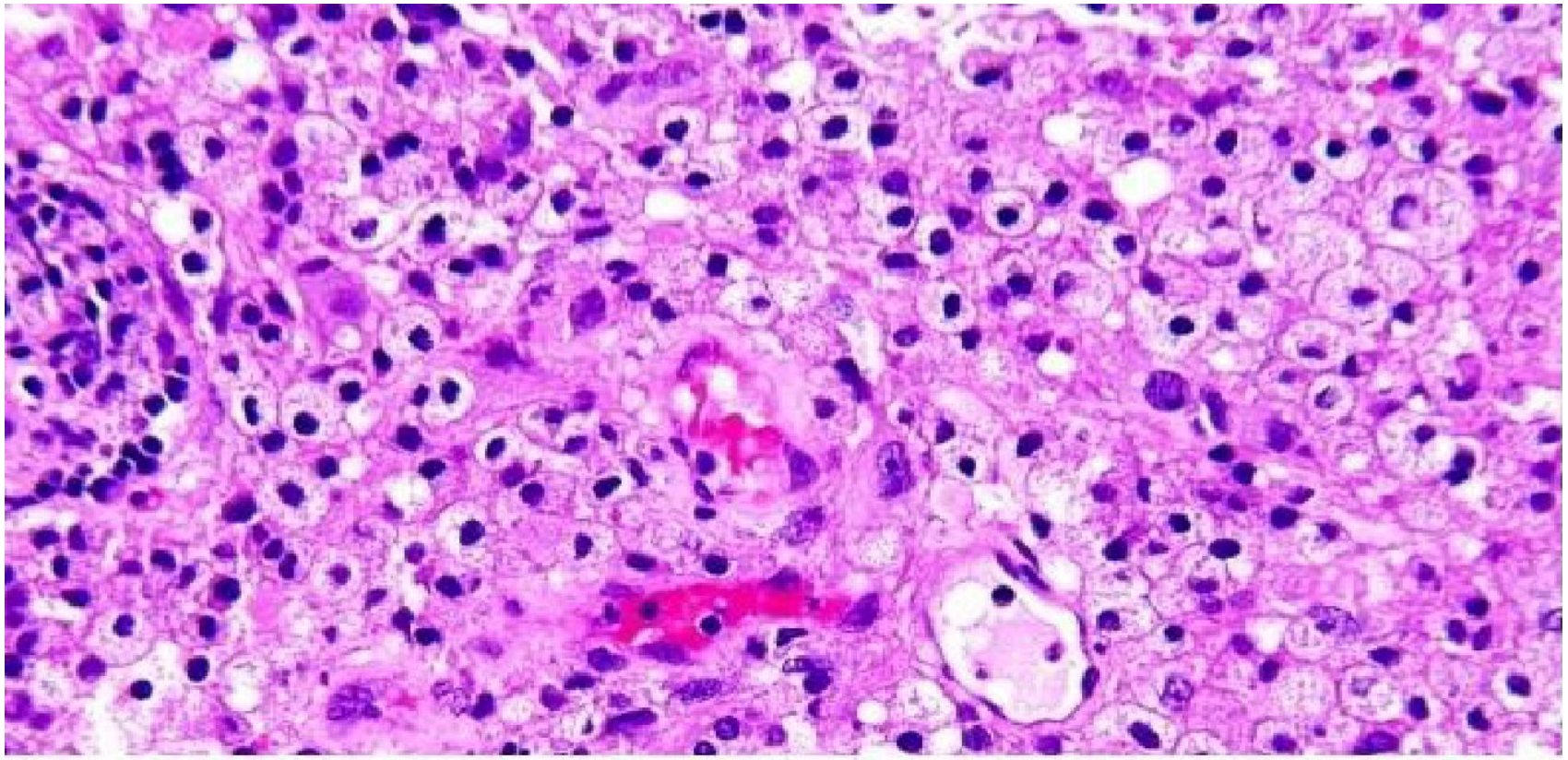
•Function: Instrument for thin sectioning of tissues.

Classification of Stains

- Acid stains (in an acid dye the basic component is colored) stains cytoplasm
- Basic stains (In a basic dye the acid component is colored) stains nucleus

Hematoxylin and Eosin (H&H):

- Hematoxylin basic like dye which stains acid molecules (such as Nucleic acids
- Eosin acidic dye which stains basic molecules (such as Cytoplasm& proteins



Hematoxylin and Eosin Stain result

What is Rotary Microtome

Microtome is a specialized precision instrument used to cut extremely thin slices or sections of tissue, known as " for microscopic examination These thin sections are necessary to observe the detailed structure of tissues, cells, and organs under a microscope, especially for histological studies

Rotary Microtome

Procedure and applications of Rotary Microtome

1-Embedding The tissue sample is embedded in a solid medium like paraffin or resin

2-.Mounting The embedded tissue block is mounted on the microtome's specimen holder

3-Sectioning The microtome cuts thin sections by moving the tissue block against the blade

4.-Collection The thin sections are collected on glass slides and stained for microscopic observation

1.Precision and Consistency

- Allows for precise control over section thickness, producing uniform and consistent sections essential for accurate histological analysis

2.Versatilit

- Can be used with a variety of embedding media, such as paraffin, making it suitable for a wide range of tissue types

3.Efficiency

- Capable of cutting multiple sections in quick succession, enhancing workflow efficiency in busy laboratories

References

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- .

Any questons?